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DETECTION OF TYPE E BOTULIN TOXIN IN AN ORGANISM

[Following is the translation of an article by T. I. Sergeyeva, Gamaleya Institute of Epidemiology and Microbiology, AMN, USSR, published in the Russian-language periodical Zhurnal Mikrobiologii, Epidemiologii i Immunobiologii (Journal of Microbiology, Epidemiology and Immunobiology) No 4, 1966, pages 54-59. It was submitted on 7 Dec 1964. Translation performed by Sp/7 Charles T. Ostertag, Jr.]

The aim of the present investigation was clearing up a number of problems relating to the pathogenetic effect of Cl. botulinum type E toxin in connection with the capability, characteristic for this microbe, of producing a protoxin which under the influence of proteolytic enzymes (pancreatin, trypsin, proteases of certain bacteria) transforms into a toxin (Matveyev and Bulatova, 1955; Sakaguchi and Tohyama, 1955; Duff et al., 1956).

In order to determine the effect of the toxin and cultures of type E botulism causative agents on animals following their penetration into the organism by various routes, and also to establish the influence of the preliminary activation of the toxin with enzymes on the detection of it in an organism, we performed a titration of the minimal lethal doses of toxin and cultures of Cl. botulinum for guinea pigs and rabbits following subcutaneous, peroral and intranasal infection. The animals received a 6-day broth culture of Cl. botulinum type E No 188-20 and sterile liquid toxin. Parallel with this we injected the same material, activated with pancreatin or trypsin by the method described by Turner and Rodwell (1943), Matveyev and Bychenko (1960). The index of activation -- the ratio of the power of the toxin after activation to its power prior to activation -- equaled 20-25.

During either method of administration the minimum amount of toxin (in Dlm for mice following intravenous administration), from which the animals died on the 3--4th day, was accepted as 1 Dlm for guinea pigs or rabbits.

Following administration under or within the skin, the minimum lethal dose of a culture for guinea pigs contained 1½--2 times less the Dlm for mice than a dose of sterile toxins of this microbe. This testified to the additional formation of toxin following the multiplication of bacterial cells in the organism of an animal. Following intranasal administration the culture and the toxin caused the death of the animals in the same doses (the same number of Dlm for mice). This indicated the uniqueness of conditions existing in the respiratory tract for the causative agent of type E botulism. This also conditioned the peculiar, still unstudied, regularities of their influence on the organism. Following subcutaneous infection of guinea pigs the lethal doses of toxin and culture, taken before and after

activation, were expressed by the same number of Dlm for mice and comprised 75 Dlm (for culture) and 100 Dlm (for toxin). During infection of rabbits the lethal doses were expressed correspondingly with 500 and 600 Dlm. Consequently, during this method of infection the pathologic effect was conditioned by active toxin which had entered the organism.

During internal administration one lethal dose of culture or toxin of type E for guinea pigs equaled correspondingly 500 and 1,000 Dlm (for mice), while for activated preparations it comprised 10,000 and 20,000 Dlm (for mice). On this basis it can be proposed that in the digestive tract of guinea pigs, as a result of the activating effect of proteolytic enzymes, an increase took place in the toxicity of native culture or toxin, observed adequately in vitro under the influence of pancreatin; therefore one lethal dose of nonactivated preparation for guinea pigs was expressed in a 20 time lesser amount of Dlm (for mice) than activated.

In the tests on rabbits we were not able to determine the peroral lethal dose; even with the administration of 10,000--15,000 Dlm (for mice) of native culture and 200,000--300,000 Dlm (for mice) of activated toxin the animals remained alive, and they had no symptoms of botulism. This speaks for the weak sensitivity of rabbits to E toxin when it is administered internally. We were not able to introduce larger doses due to the absence of a more active type E toxin.

During the intranasal administration of activated and nonactivated toxins and cultures of type E, apparently no activation of prototoxin in the organism took place, since the activated and nonactivated preparations caused the death of the animals in the same doses, equalling 100-150 (for mice) - 1 Dlm for pigs and 1,000 Dlm (for mice) - 1 Dlm for rabbits.

Having made a recalculation of the amount of toxin in a Dlm (for mice) per one kg of animal weight, one can be satisfied that guinea pigs and rabbits display almost the same sensitivity to type E botulin toxin after it has been administered subcutaneously and intranasally, while when this toxin was administered internally the rabbits turned out to be mildly susceptible.

In order to clear up the influence of the preliminary activation of toxin or a culture of type E botulism causative agent on the frequency of detecting it in the organs of animals, guinea pigs were administered internally similar doses (for these animals) of activated and nonactivated preparations (cultures or toxins). The contents of the stomach, small intestines and liver were taken from sacrificed animals and they were subjected to an investigation for the presence of toxin with the help of the neutraliztion reaction on white mice with type E specific serum.

When guinea pigs were infected with 2 Dlm of a native culture the toxin was detected in the small intestines of 4 out of 6 test animals (table 1). When the dose was doubled the toxin was found in the small intestines in all cases and in the liver in half of the cases, however the toxin was found in

the stomach of only one pig. Attention was merited by the fact that following infection with 4 Dlm of a pancreatin activated culture the toxin was found with the same frequency in the stomach and small intestines of dead pigs, whereas following the administration of the same dose of nonactivated culture the toxin was detected in the stomach rarely when it was present in the small intestine.

The investigation of the organs of guinea pigs infected with activated and nonactivated type E botulin toxin showed that for detecting the toxin in the small intestines of dead animals it is necessary to administer them twice the quantity of lethal doses than during infection with the culture, which is apparently connected with the additional formation of toxin during the multiplication of microbes in the intestines.

In these tests, just as in the preceding ones, the activated toxin was exposed earlier in the small intestine and the stomach, and the nonactivated toxin -- mainly in the contents of the small intestine. Consequently, the possibility of detecting toxin in the small intestines of pigs did not depend on whether native or activated preparations were administered, since in both cases when the doses were the same the toxin was found with the same frequency.

The detection of toxin with the same frequency in the stomach and the intestines of animals infected with activated preparations may be explained by the fact that here toxin was exposed which entered the organism and not which was formed as the result of the in vivo activation of protoxin. All this testifies that the activation of toxin took place in the intestines of infected animals. As a result of this the destructive effect of the contents of the intestines on white mice was intensified and this had an effect on the results of the investigation.

It was pointed out above that when botulinum toxin or a culture of type E was administered through the nose into the respiratory tracts of animals, no differences were noted in the pathologic effect of native and activated preparations. Therefore in the experiments on intranasal administration we used only native toxin and culture.

Type E toxin, administered to guinea pigs throught the nose in a "pure" form and with microbial bodies, was detected in the lungs most of the time following infection of the animals with 2 Dlm (table 2). Toxin was determined in the blood and liver of dead animals only in those cases when infection was carried out with 5 Dlm.

In guinea pigs infected through the mouth or through the nose with 0.75, 1, and 2 Dlm of toxin or type E culture, the blood was investigated for the presence of toxin in 1, 2, 4, 24, 48 and 72 hours after infection. For the detection of toxin in the blood we used the method of phagocytic index determination (Minervin, 1961), and in a number of tests parallelly -- the neutralization reaction on white mice.

Toxin was not detected in the blood of guinea pigs infected through the mouth with 0.75 Dlm (table 3). In the guinea pigs which had received 1 or 2 Dlm of culture the toxin was exposed in the blood in 4, 24 and 48 hours after infection, while following the administration of sterile toxin it was determined in the blood of the animals primarily after 4 hours from the moment of contamination. Toxin was not revealed in blood taken from pigs in 1 and 2 hours after the administration of the toxic material.

The method of calculating the phagocytic indices when investigating the blood of infected guinea pigs turned out to be somewhat more sensitive than the neutralization reaction, though sometimes results were obtained which were not sufficiently conclusive.

It can be seen from table 4 that in 2 pigs out of 3, infected intranasally with 0.75 Dlm of culture, the toxin was found in 4 hours by the phagocytic method, while the result of the neutralization reaction in these pigs was negative. When 1 and 2 Dlm of culture was administered to these animals through the nose the toxin was detected in the blood in 2, 4 and 24 hours after infection.

When guinea pigs were infected through the nose with the same doses of sterile native toxin the toxin was also determined in the blood of the animals; with the help of the method of phagocytosis suppression the toxin was detected after 2, 4 and 24 hours after the administration of 1 and 2 Dlm.

A comparison of the data obtained with the results of investigating the organs and blood of guinea pigs infected through the mouth and through the nose with type A, B and C botulinum toxin (Sergeyeva, 1962, 1963) showed that type E toxin was detected in the gastro-intestinal tract of the animals following the administration of 4--6 times less "peroral" lethal doses in comparison with doses of other toxins. This difference may be explained, on the one hand, by an intensification of the action of type E toxin in vivo under the influence of digestive enzymes in the intestines, and on the other hand - the exceedingly high resistance of this toxin to changes in the reaction of the medium of the gastro-intestinal tract.

When determining type E botulinum toxin in the organs of animals sacrificed following aspiration infection the data obtained were basically the same as for animals infected with type A, B and C toxins; in all cases the toxin was found in the lungs after the intranasal administration of 2--5 lethal, for the particular species of animal, doses of culture or toxin.

Conclusions

1. Guinea pigs and rabbits displayed a similar sensitivity to the subcutaneous and intranasal administration of type E Cl. botulinum toxin; however, during peroral infection the rabbits were mildly susceptible.

2. Following the internal administration of the toxin or culture of type E botulsim causative agent to animals ~~we noted~~ the activation of prototoxin under the influence of proteolytic enzymes in the intestines. When infection with toxin or culture was through the nose a similar activation was not observed.

3. As a result of activation, type E botulin toxin was detected in the small intestine earlier than in the other organs following the administration of comparatively small doses.

4. When the animals were infected through the mouth, type E botulin toxin was detected in the blood for a more or less prolonged period of time, depending on whether the toxin was administered with or without microbial bodies.

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Table 3

Detection of type E botulin toxin by the method of phagocytosis suppression in the blood of guinea pigs infected through the mouth

Dose of toxin (Dlm)	Preparation	Number of pigs	Results of the investigation after infection, in					
			1 hr	2 hrs	4 hrs	24 hrs	48 hrs	72 hrs
0.75	Toxin	2	2/0	2/0	2/0	2/0	2/0	2/0
	Culture	3	3/0	3/0	3/0	3/0	3/0	3/0
1	Toxin	3	3/0	3/0	3/3	3/1	3/0	3/0
	Culture	4	4/0	4/1	4/4	4/3	4/3	4/0
2	Toxin	3	3/0	3/0	3/3	3/2	3/1	--

Table 4

Detection of type E botulin toxin by the method of phagocytosis suppression in the blood of guinea pigs infected intranasally

Dose of toxin (Dlm)	Preparation	Number of pigs	Results of the investigation after infection, in					
			1 hr	2 hrs	4 hrs	24 hrs	48 hrs	72 hrs
0.75	Toxin	2	2/0	2/0	2/0	2/0	2/0	2/0
	Culture	3	3/0	3/0	3/2	3/0	3/0	3/0
1	Toxin	3	3/0	3/1	3/3	3/2	3/0	3/0
	Culture	3	3/0	3/2	3/3	3/3	3/0	--
2	Toxin	2	2/0	2/1	2/2	2/1	--	--
	Culture	2	2/0	2/2	2/2	2/2	--	--

Investigation of the organs of guinea pigs destroyed after the internal administration of a various number of lethal doses (for guinea pigs following peroral infection)

Preparation tested	Dose of toxin (Dlm)	Preparation administered	Number of animals	Period of death after administration of toxin (in hours)	Result of investigation		
					liver	stomach	small intestine
Native	1	Toxin	5	84--96	5/0	5/0	5/0
		Culture	6		6/0	6/0	6/0
	2	Toxin	4	48--72	4/0	4/0	4/1
		Culture	6		6/0	6/0	6/4
	4	Toxin	4	36--48	4/0	4/0	4/4
		Culture	4		4/2	4/1	4/4
After activation	1	Toxin	4	84--96	4/0	4/0	4/0
		Culture	5		5/0	5/0	5/0
	2	Toxin	4	48--72	4/0	4/0	4/0
		Culture	6		6/0	6/1	6/4
	4	Toxin	4	36--48	4/0	4/3	4/4
		Culture	4		4/2	4/4	4/4

Note: Here and subsequently - numerator = number of tests, denominator = number of tests in which toxin isolated.

Table 2

Investigation of the organs of guinea pigs destroyed after intranasal infection with a various number of minimum lethal doses (for guinea pigs during this method of infection)

Dose of toxin (Dlm)	Preparation administered	Number of animals	Period of death after administration (in hours)	Result of investigation		
				Liver	Blood	Lungs
1	Toxin	5	72--96	3/0	3/0	3/0
	Culture	4		4/0	4/0	4/0
2	Toxin	3	36--48	3/0	3/1	3/2
	Culture	4		4/0	4/1	4/3
5	Toxin	3	24--36	3/1	3/2	3/3
	Culture	4		4/1	4/3	4/4